PARASITOLOGICAL DIAGNOSIS OF MUCOCUTANEOUS LEISHMANIASIS DUE TO \textit{LEISHMANIA B. BRAZILIENSIS} IN BOLIVIA

Laure Dimier-David, Christophe David, Pierre Ravisse, Renato Bustillos, Susana Revollo, Philippe Lyèvre, Maruschka Muñoz, Fernando Vargas and Jean-Pierre Dedet

SUMMARY

Parasitological diagnosis, using stained smears, culture and pathological examination of biopsy, was studied in 146 patients infected with mucocutaneous leishmaniasis, in Bolivia and Peru. The most efficient parasite detecting technique appeared to be the smear examination in cutaneous lesions (33\% positive) and the pathology in case of mucous lesions (28\% positive). In both, cutaneous and mucous lesions, the parasites were found most frequently in old lesions.


MATERIAL AND METHODS

The study was realized within a sample of 146 patients which were seen at the parasitology department of the Instituto Boliviano de Biologia de Altura (La Paz, Bolivia) between 1988 and 1990. They originated from the endemic areas neighbouring La Paz (Yungas, Alto Beni and Beni in Bolivia, Puno and Madre de Dios departments in Peru).

For each patient, the search for parasite was simultaneously conducted using the three following techniques:
1. smears made with material obtained from scratching the cutaneous lesion periphery or by imprints of biopsy section in case of mucous lesion. The smears were fixed by methanol and Giemsa stained;
2. culture inoculation on biphasic NNN medium containing Schneider medium and antibiotics as the liquid phase, of material obtained from scratching or biopsying the lesion. The isolates obtained were then characterized by isoenzyme electrophoresis;
3. pathological examination of biopsy material. The biopsy material was taken with a 4 mm punch biopsy on the edge of the cutaneous lesion or with a cutting biopsy forceps in the case of mucous lesion, and

Instituto Boliviano de Biologia de Altura, c/o Embajada de Francia and Servicio de Otorrinolaringología, Hospital das Clínicas, La Paz, Bolivia. Unité d’Histopathologie, Institut Pasteur, Paris, France.

Address to correspondence: Dr. Jean-Pierre Dedet, Laboratoire d’Ecologie Médicale et Parasitologie Parasitaire, Annexe de la Faculté de Médecine, 163 rue Auguste Broussonet, 34000 Montpellier France.

Received para publicação em 01/07/91.
fixed in Bouin's fluid or 10% buffered formalin. The sections were stained with haemalum-eosin-safranine or Giemsa's stain.

The immunological investigation realized in each patient included: leishmanin skin testing and detection of circulating antibodies by indirect immunofluorescence.

RESULTS

The patients were divided into 2 groups, according to the type of lesion (cutaneous or mucosal) they harboured.

Patients with cutaneous lesions

This group included 30 patients harbouring only cutaneous lesions. Of them, 23 were males and 7 females. The age range was 25.7 ± 3.2 years. Three originated from Peru, the others were Bolivian.

The lesions were ulcerative, with marked inflammatory fringe. The lesions were unique in 36.6% of the patients and multiples in 63.3%. They were localized on lower limbs in 53.8% of the cases and 86.6% of them had a duration period inferior to one year.

The parasite was found in 15 patients (50%) according to the following identification techniques:

- positivity of smear alone: 2 cases (13.3%)
- positivity of culture alone: 3 cases (20.0%)
- positivity of pathology alone: 2 cases (13.3%)
- positivity of both smear and culture: 2 cases (13.3%)
- positivity of both smear and pathology: 5 cases (33.3%)
- positivity of smear, culture and pathology: 1 case (6.6%)

Table 1 shows the comparisons of the three identification techniques.

The six isolates obtained in culture were characterized by isoenzyme electrophoresis as *L. b. braziliensis*.

According to the duration period of the lesions, the parasite was found in 40% of the lesions of less than 1 year of evolution and in 75% of the lesions of more than 1 year of evolution.

The leishmanin skin test was positive in 82.1% and the IFI result was included between 1/40 and 1/80 in 86.6% of the cases.

Patients with mucosal lesions

The second group of patients included 116 patients affected with mucosal leishmaniasis. One hundred nine were males and seven females. The age range was 3.7 to 38.5 years. Fifteen of them originated from Peru, the others were Bolivians.

The evolution time of mucous involvement was less than 1 year in 12%, between 1 and 5 years in 58.6%, between 5 and 10 years in 21.5%, between 10 and 20 years in 6% and more than 20 years in 1.7% of the cases. The extension of the mucosal involvement was limited (nose + palate mucosa) in 38.7%, important (nose + palate mucosa + pharynx or larynx lesion) in 28.4% and very important (all the mucous of the otorhinolaryngeal (ORL) sphere) in 32.7% of the cases.

The parasite was found in 60 patients (51.7%) according to the following identification techniques:

- positivity of smear alone: 10 cases (8.6%)
- positivity of culture alone: 15 cases (12.9%)
- positivity of pathology alone: 18 cases (15.5%)
- positivity of both smear and culture: 2 cases (1.7%)
- positivity of both smear and pathology: 10 cases (8.6%)
- positivity of smear, culture and pathology: 5 cases (4.3%)

The Table 1 shows the comparisons of the three examination techniques in the two groups of patients.

The 15 isolates obtained from the 22 positive cultures were characterized as *L. b. braziliensis* by isoenzyme electrophoresis.

Regarding the evolution time of the lesions, the parasite was found in 48.7% of the lesions of less than 5 years of evolution and in 58.8% of the lesions of more than 5 years of evolution.

The leishmanin skin test was positive in 75% and the IFI results included between 1/80 and 1/160 in 70.6% of the cases.
DISCUSSION

The results of our study showed that using or techniques the percentage parasite positive patients in cutaneous and mucosal lesions of *L. b. braziliensis* leishmaniasis in Bolivia are similar: 50% in cutaneous lesions versus 51.7% in mucous lesions.

Compared to literature data these percentages appear weak in the case of cutaneous lesions: Cuba et al. detected parasites in 71.2% of cutaneous lesions of the same mucocutaneous leishmaniasis in Três Baços (Bahia, Brasil). Also in Bolivia, Desjeux et al. obtained 48.6% positive results in parasite detection by culture and hamster inoculation in a sample of 72 cutaneous lesions biopsied from mucocutaneous leishmaniasis. In a 216 patients sample observed in French Guyana, smear and culture detection of the parasite were positive en 61.6% of the cutaneous lesions due to *L. b. guyanensis* (Dedet, unpublished data).

In respect to the mucous lesion, the positive percentage is equivalent to that of Cuba et al. who observed 48% of positivity in mucosal lesions, but differs largely from the 100% positivity obtained in a limited sample of patients in Colombia by Saravia et al.

According to our results, the most efficient parasite detecting techniques appeared to be the smears in cutaneous lesions (33.3% of positivity) and the pathology in case of mucous lesions (28.4%). For logistic reasons, hamster inoculation was not done although this is an efficient method of diagnosis. Our observation stresses the value of routine pathological method in the diagnosis of mucocutaneous leishmaniasis. This technique not only can give direct evidence of the parasite, as reported here, but can also allow the elimination of other pathologies as will be discussed in detail elsewhere.

In our experience, the parasite was found more frequently in the cases of important and very important mucous extension (57.7% positivity) than in the cases of limited involvement (42.2% positivity). Marsden observed in the same way that it was “easier to detect parasites in multiple mucosal lesions than in single ones”.

Another paradoxical data evidenced by our observation is that in both cutaneous and mucous lesions the parasites were found most frequently in old lesions (more than one year evolution for cutaneous lesions and more than 5 years evolution for mucous lesions) than in recent lesions, to the

contrary of other authors' observations1410. These observation illustrate the importance of further comparative studies, in order to show the variations due to local situations, both in lesion expression and in laboratory conditions.

RESUMO

Diagnóstico parasitológico, usando esfregaço, cultura e exame anatomopatológico de biópsia, foi estudado em 146 pacientes infectados com leishmaniose mucocutânea, na Bolívia e Peru. A mais eficiente técnica de detectar parasitas parece ser o exame de esfregaço para lesões cutâneas (33% de positividade) e anatomopatológico para lesões de mucosas (28% de positividade). Em ambas lesões, cutânea e mucosa, parasitas foram mais frequentemente encontrados em velhas lesões.


ACKNOWLEDGEMENTS

We thank Pr. Fernando Cardenas and Pr. Luis Valda of the department of Dermatology, Hospital de Clínicas of La Paz, were the patients were hospitalised. The Instituto Boliviano de Biologia de Altura receives a support from the Ministère des Affaires Etrangères (Paris, France), the Ministerio de Previsión Social y Salud Pública and the Universidad Mayor de San Andrés (La Paz, Bolivia).

REFERENCES